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Local Minocycline to Reduce Future Inflammation and Bone Loss in Periodontal Maintenance Patients

Jeffery S. Jensen
University of Nebraska Medical Center

LOCAL MINOCYCLINE TO REDUCE FUTURE INFLAMMATION AND BONE LOSS IN PERIODONTAL MAINTENANCE PATIENTS

by

Jeffery S. Jensen, D.D.S.

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Advisory Committee:

Matthew R. Byarlay, D.D.S., M.S.

Thomas M. Petro, Ph.D.

Amy C. Killeen, D.D.S., M.S.

Eric Y.K. Fung, Ph.D.

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The decision to seek a master's degree during my periodontal residency was one that I made before applying for positions. My experience with periodontics in dental school gave me the desire to be involved in the research field and to give back to the profession. However, I was inexperienced in regards to conducting research. I was fortunate enough to receive the support I needed in order achieve my goals of working on a solid, strong project. During my residency, I have had the opportunity to read a great deal of literature pertaining to the field of periodontics, and it has been a wonderful opportunity to work towards expanding the information available to the community. My thanks go out to all of those who have provided support and guidance along this journey.

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LOCAL MINOCYCLINE TO REDUCE FUTURE INFLAMMATION AND BONE LOSS IN PERIODONTAL MAINTENANCE PATIENTS

Jeffery S. Jensen, D.D.S., M.S.

University of Nebraska, 2016

Advisor: Richard A. Reinhardt, D.D.S., Ph.D.

The objectives of this study were to compare the effect of SRP+MM versus SRP alone on inflammatory cytokines, interleukin (IL)-1β and IL-1 receptor antagonist (ra), found in gingival crevicular fluid (GCF) and saliva, and correlate IL-1 values with clinical outcomes over a 2-year period of PMT. Fifty-five patients with an initial posterior pocket ≥5 mm and history of bleeding on probing (BOP) were randomized to test (SRP+MM, n=27) and control (SRP, n=28) groups. Clinical measurements and GCF samples were done at baseline and 24 months. SRP+MM or SRP were provided at 6-month intervals. IL-1β and IL-1ra were measured in GCF and saliva (24 months). A total of 48 patients completed the study (SRP+MM, n=23; SRP, n=25). Groups were compared using t-test, Wilcoxon test, and Spearman correlations. There was no significant difference or change in IL-1 or IL-1β/IL-1ra ratios levels between groups after 24 months. BOP level decreased significantly in both groups (SRP+MM=56%, SRP=45%). Bone loss was not significantly altered in either group. Both groups showed significant improvements in probing depth (p=0.001) and clinical attachment level (p=0.001). Baseline GCF IL-1β was correlated with bone loss at 24 months and salivary IL-1β was correlated with increase in probing depth at 24 months ($r=0.34$, $p\leq0.03$). The addition of MM to SRP during PMT did not significantly affect IL-1 levels or clinical parameters.

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BOP	bleeding on probing
CAL	clinical attachment level
ELISA	enzyme-linked immunosorbent assay
GCF	gingival crevicular fluid
IBL	interproximal bone loss
IL-1β	interleukin-1 beta
IL-1ra	interleukin-1 receptor antagonist
MM	locally-delivered minocycline microspheres
PD	probing depth
PMT	periodontal maintenance therapy (control)
REC	gingival recession
SRP	scaling and root planing

CHAPTER 1: INTRODUCTION

Periodontitis is an inflammatory disease affecting the supporting structures of the dentition. Both the hard and soft tissues of the periodontium are affected by the disease (AAP Parameters of Care 2000). The periodontium consists of several different tissues, including: cementum, alveolar bone, and the periodontal ligament. Periodontal disease affects up to half of the population of the United States over the age of 30; a figure much larger than originally estimated (Eke et al. 2015). Aging also appears to play a role in the decrease of clinical attachment levels and prevalence of the disease with a higher rate of occurrence in males (Eke et al. 2015).

Treatment of periodontitis with debridement of bacterial biofilm, removal of calculus and contaminated cementum through scaling and root planing (SRP) and/or surgical treatment of periodontal soft tissue and bone has been shown to be relatively successful with resolution of inflammation and improvement of clinical parameters (Kaldahl et al. 1996a, Becker et al. 2001, Mialoa et al. 2015). When a patient is determined to be stable following non-surgical and/or surgical therapy, they are placed into a periodontal maintenance therapy (PMT) schedule. Periodontal pockets that continue to show bleeding on probing (BOP) are indicative of continued inflammation (Amato et al. 1986) and presence of bacteria (Wilson et al. 2008). If recurrence of disease is noted during PMT, treatment with additional SRP may be prescribed and has been shown to be effective (Kaldahl et al. 1996b).

Like any other medical treatment, not all patients respond in the same way to periodontal treatment. In light of these varied responses, adjunct therapies to SRP have been developed and employed. These adjuncts include systemic antibiotics (Sgolastra et al. 2014), local delivery of antibiotics (Kinane & Radvar 1999), subgingival irrigation (Jolkovsky et al. 1990),

and lasers (Cobb 2006). Each of these therapies has been used with varying degrees of success and efficacy. Adjunct periodontal therapies aim to reduce the microbial and inflammatory load to a level where successful healing can occur. Local administration of minocycline microspheres (MM) is a technique widely used. Evidence for use of minocycline in conjunction with SRP exists (Williams et al. 2001), however evidence is lacking in the use of minocycline in periodontal maintenance therapy.

CHAPTER 2: LITERATURE REVIEW: PERIODONTITIS

Periodontitis is a disease affecting the supporting tissues of the dentition. A patient presenting with periodontitis may have manifestations of gingival erythema, discomfort, increased amount of gingival crevicular fluid (GCF), increase probing depths (PD), increased loss of clinical attachment level (CAL), tooth mobility, bleeding on probing (BOP), suppuration, abscesses, and gingival recession (REC). Treatment of the disease is imperative because failure to do so may result increased loss of attachment and eventual tooth loss (AAP Position Paper 1999).

Bacterial plaque and calculus have been identified as the main etiology of chronic periodontitis (Socransky & Haffajee 1992). However, the mere presence of pathogens does not appear to be sufficient for progression of the disease. Disease may occur when the pathogen is present and it is of a virulent clonal type, it possesses the chromosomal and extra-chromosomal genetic factors to initiate disease, the host is susceptible to the pathogen, the pathogen is present in numbers sufficient to exceed the threshold for the host, the pathogen is located in the right place, other bacteria species foster or do not hinder the progress of the disease, and the local environment is conducive to the expression of the species' virulence properties (Socransky & Haffajee 1992). Host susceptibility plays a vital role in the progression of periodontal disease. Genetic factors such as neutrophil dysfunction (Van Dyke et al. 1985), neutrophil hyperactivity (Matthews et al. 2007), and IL-1 polymorphism (Kornman et al. 1997) have been suggested as possible genetic factors contributing to periodontal disease progression.

When treating periodontal disease, a clinician attempts to resolve inflammation, prevent progression of periodontal destruction, and restore the patient to comfort and function. These goals may be achieved through different therapy, including non-surgical periodontal

therapy, surgical therapy, or a combination of both. These therapies aim to disrupt the bacterial biofilm, reduce microbial load, and minimize future recolonization. Outcomes of this treatment are measured using PD, BOP, PI, and gain in CAL. (Haffajee et al. 1997)

When performing non-surgical periodontal therapy, teeth are debrided through scaling and root planing. Scaling and root planing are normally done using hand instruments and/or ultrasonic scalers. During instrumentation of the teeth, plaque and calculus are removed, subgingival bacterial load is reduced (Socransky et al. 2013), and the roots are detoxified (Nishimine & O'Leary 1979). Scaling and root planing has long been held up as the "gold standard" of periodontal treatment (Cobb 2002). Following scaling and root planing, one can expect to see improvements in periodontal health when measuring PD, BOP, PI, and CAL (Kaldahl et al. 1996a, Becker et al. 2001, Mialoa et al. 2015). Other factors may be found to influence periodontal disease, such as: occlusal trauma, iatrogenic restorations (Jeffcoat 1980), tooth crowding (Bollen 2008), and smoking (Albandar 2000). In addition to non-surgical therapy, some cases of periodontal disease may need surgical therapy.

Following non-surgical and/or surgical therapy of periodontitis, patients that are deemed to be stable by their clinician are placed into PMT. PMT has been shown to be critical to long-term success (Nyman et al. 1975, Ramfjord 1982, Becker et al. 1984a, Becker et al. 1984b). Evidence is present that patients who refuse to follow-up with periodontal maintenance return to a diseased state (Axelsson & Lindhe 1981, Becker et al 1984a, Becker et al 1984b). The goals of periodontal maintenance include: 1) prevention or minimization of recurrence of disease progression in patients who were previously treated for periodontitis, 2) prevention or reduction of the incidence of tooth or implant loss by monitoring the dentition, and 3) to increase the probability of locating and treating other conditions or diseases found within the

oral cavity in a timely manner (Cohen 2003). During each PMT appointment, conditions of the periodontium are measured and evaluated, daily home care is reviewed and reinforced, and periodontal treatment is performed as needed (Cohen 2003). Sites that have residual signs of inflammation after and during PMT have been shown to be more likely to progress and deserve additional treatment (Claffey et al. 1990). PMT is generally carried out at 3-4 month intervals (Nyman et al. 1975, Schallhorn & Snider 1981).

CHAPTER 3: LITERATURE REVIEW: LOCAL DELIVERY OF MINOCYCLINE

Minocycline is a broad-spectrum antibiotic in the tetracycline family and has a bacteriostatic effect. In addition to the antibacterial effects, minocycline also appears to exert an anti-inflammatory effect in the body. Minocycline has been shown to inhibit the activity of matrix metalloproteinases, inducible nitric oxide synthase, and cyclooxygenase-2 (Chen et al. 2000; Yrjänheikki et al. 1999). IL-1β and tumor necrosis factor α (TNF-α) production are also impaired by minocycline (Suk 2004). Minocycline also has been shown to inhibit the inflammatory response of monocytes challenged by LPS (Pang et al. 2012) and the formation of osteoclasts (Holmes et al. 2004). Minocycline's combined anti-bacterial and anti-inflammatory effects make the drug an appealing therapeutic option in the treatment of periodontal disease.

Systemic antibiotics have been utilized in the treatment of periodontitis; however, the widespread use of them has been discouraged due to the possibility of developing resistant organisms. The development of locally delivered antibiotics has come about in hopes of avoiding the undesirable effects of systemic antibiotic administration. To be clinically effective, locally delivered antibiotics must fulfill several criteria. These therapies must reach the targeted site of action, remain at an effective concentration, and last for an adequate period of time (da Rocha et al. 2015).

Minocycline often is used as an adjunct to SRP through a locally-delivered mechanism. Minocycline is microencapsulated in polyglycolide-co-dl lactide, a bioabsorbable polymer. The encapsulated minocycline is then administered into the periodontal pocket in a powder form. Immediately following contact with moisture, the polymer begins to hydrolyze and release the minocycline. Administration of the minocycline microspheres results in a localized, sustained release of the antibiotic at concentrations of 340 µg per ml in GCF at 14 days (Williams et al.

2001). These concentrations are much higher than the minimum inhibitory concentrations for periodontal pathogens, normally thought to be 1-2 µg per ml (Hagiwara et al. 1998).

Several studies have investigated the application of minocycline microspheres during initial SRP. Williams et al. (2001) conducted a multi-center clinical trial investigating the efficacy and safety of locally administered minocycline microspheres. The study included 748 patients diagnosed with moderate to severe periodontitis randomized into three groups: SRP alone, SRP plus vehicle, and SRP plus minocycline microspheres. The authors reported that SRP plus minocycline microspheres provided more probing depth reduction when compared to SRP alone and SRP plus vehicle. This difference was statistically significant at 1 month and was maintained throughout the 9 months of follow-up. Several additional studies have shown that when used in adjunct to initial SRP, application of MM has resulted in improved clinical parameters (Paquette et al. 2004; Lessem and Hanlon 2004).

Oringer tested the ability of minocycline microspheres to effect markers of bone resorption. GCF samples were obtained at baseline, 1, 3, and 6 months and clinical measurements were recorded. Pyridinoline cross-linked carboxy-terminal telopeptide of type I collagen (ICTP), a bone-specific degradation product, and IL-1 were measured from GCF samples. Patients also received SRP in addition to MM or a vehicle control. Authors determined that GCF levels of ICTP and IL-1 were significantly reduced in patients receiving MM along with SRP (Oringer et al. 2002).

Goodson et al. (2007) investigated the antimicrobial effects of minocycline microspheres when used as an adjunct to SRP. Specifically, the authors looked at the effects of MM on the levels of red-complex bacteria: *Porphyromonas gingivalis*, *Tannerella forsythia*, and *Treponema denticola.* Test and control sites were sampled at baseline and 30 days. The study concluded

that levels of red complex bacteria were significantly more decreased in test sites than control sites. Clinical parameters also showed a significant improvement in the test group when compared to the control group (PD, CAL, BOP). Additional studies have also reported a reduction in red complex bacteria when using MM in conjunction with SRP (Grossi 2007; Bland et al. 2010).

Currently, minocycline microspheres are approved for placement in inflamed periodontal pockets in conjunction with initial SRP therapy. However, MM is commonly used to treat residual inflamed periodontal pockets following active therapy. A small number of investigations have been completed observing the effect of MM in patients undergoing PMT.

Meinberg et al. (2002) studied the difference in clinical parameters between conventional periodontal maintenance and SRP with MM. The authors concluded that SRP and MM resulted in PD reduction and less frequent radiographic bone loss height than in conventional periodontal maintenance. MM was applied at baseline, 1, 3, and 6 month appointments following SRP at the baseline appointment only. Control patients received conventional PMT at 3 month intervals for 1 year.

An additional study, van Steenberghe et al. (1999), evaluated the clinical and microbiological outcomes of repeated application of minocycline gel subgingivally. SRP was done at baseline, 6, and 12 months. Minocycline gel was applied at baseline, 2 weeks, 1, 3, 6, 9, and 12 months. The authors concluded that repeated application of subgingival minocycline gel resulted in improvement in both clinical and microbiologic variables over 15-months when compared to SRP alone.

Long-term studies involving the measurement of clinical parameters and inflammatory biomarkers during PMT appear to be lacking in the literature. This investigation attempts to

determine whether repeated application of MM at 6-month intervals along with PMT will improve clinical parameters and decrease the level of inflammatory biomarkers when compared with 6-month PMT alone over a 2 year period.

CHAPTER 4: LITERATURE REVIEW: INTERLEUKEN-1β AND INTERLEUKIN-1ra

Interleukin-1β is a pro-inflammatory cytokine released during the inflammatory response, such as periodontitis, due to the presence of bacteria (Ishihara et al. 1997, Cochran 2008). IL-1β is a glucoprotein of 17 kDa released primarily from macrophages and monocytes (Mergenhagen 1984, Matsuki et al. 1992). However, IL-1β may be released from a myriad of different types of cells, including: fibroblasts, dendritic cells, Langerhans cells, B cells, endothelial cells, neutrophils, epithelial cells, and bone cells (Oppenheim et al. 1986, Horowitz 1993). IL-1β binds to interleukin-1 receptors on the cell membranes of the target cell. The production of IL-1β may be stimulated by various means, such as: microorganisms, microbial products, inflammatory agents, or antigens (Preiss & Meyle 1994). The pro-inflammatory effects of IL-1β are many. Some of these effects include: stimulation of T-lymphocytes and lymphokine production (Mizel 1987), proliferation of B-lymphocytes and antibody production (Chiplunkar et al. 1986), proliferation of fibroblasts, stimulation of prostaglandin release, enhancement of neutrophil chemotaxis and activation (Sauder et al. 1984), and release of metalloproteinases (Dinarello 1991). Perhaps most importantly in periodontal disease, IL-1β promotes osteoclast formation and is a major factor in the induction of bone demineralization (Dewhirst et al. 1985). As such, IL-1β is a mediator of tissue destruction seen in the course of periodontal disease (Page et al. 1997). Levels of IL-1β have been shown to be higher in gingival tissue involved in active periodontal sites compared to healthy periodontal sites (Stashenko 1991).

IL-1β is measured in the oral cavity in different ways, including the collection of GCF and saliva. IL-1β has been shown to be detectable in GCF samples. Masada et al. (1990) identified IL-1β in the GCF in 15 of 15 patients with untreated periodontitis. Kinane et al. (1992) were able to detect IL-1 in the GCF of 12 of 12 patients with experimental gingivitis. Gursoy et al. (2009) were able to detect IL-1β in the saliva of 165 of 165 patients regardless of their periodontal status.

When evaluating IL-1β in the GCF, the investigator may use different means of measurement, such as concentrations of IL-1β or the total amount of IL-1β in a timed sample. Gilowski et al. (2014) found that reporting on the concentration of IL-1 levels could be misleading biochemically. The total amount of IL-1 in a sample produced a more reliable result. This is due to the impact of small volumes in the denominator (GCF) causing fluctuations in concentrations. Lamster et al. (1986) reported that due to the small amount of GCF collected in each sample and the variability of sample size between individuals, using the total amount of cytokine present is more appropriate for reporting GCF data. Wei et al. (2004) were unable to show any significant correlation between clinical parameters and concentration of IL-1β, but significance was shown when using total amount of IL-1. Engebretson et al. (2002) found that both concentrations of IL-1 and total amount of IL-1 differed between groups with different severity of disease. However, the total amount of IL-1 had more pronounced differences than the concentration difference between groups.

Ishihara et al. (1997) found that as the severity of periodontal disease increased the levels of IL-1β also increased. IL-1β was not detected in periodontally healthy subjects. In diseased subjects, the IL-1β level correlated with bone loss. Engebretson et al. (2002) showed that total IL-1β in GCF correlated with severity of PD and CAL in patients with mild, moderate, or severe periodontitis. Also noted was the fact that patients with severe periodontitis exhibited an almost 2 fold increase in IL-1β in shallow PD compared to patients with mild or moderate periodontitis. The authors suggest that IL-1β expression in the GCF is a host trait, and not simply due to clinical parameters at the site. Gamonal et al. (2000) found that the amount of crevicular IL-1β was associated with periodontal status in 18 patients. IL-1β was detected at higher amounts in sites deemed active compared to sites deemed inactive. These findings suggest that total IL-1β may be a valuable marker in the GCF to determine the inflammatory activity in

periodontal tissue. Sexton et al. (2011) found that in total IL-1β in saliva correlated with periodontal disease severity in 33 patients.

SRP has repeatedly been shown to improve clinical signs of inflammation and reduce IL-1β levels in periodontally diseased areas (Engebretson et al. 1999, Gamonal et al. 2000, Engebretson et al. 2002, Konopka et al. 2012). The reduction in IL-1β levels may last up to 24 weeks following SRP before returning to comparative baseline levels (Engebretson et al. 2002). Sexton et al. (2011) showed that IL-1β was reduced in patients receiving SRP. Patients receiving oral hygiene instructions only did not show the same decrease in IL-1β levels.

Following initial therapy, patients begin receiving PMT. Pockets exhibiting continued inflammation in the form of BOP are of interest to the clinician due to the continuation of the disease process. IL-1β levels have been shown to correspond to disease activity during PMT. Reinhardt et al. (2010) investigated the relationship between IL-1β levels and attachment and bone loss in periodontal maintenance patients. The patient population consisted of postmenopausal women with moderate to advanced periodontitis. Patients receiving PMT who showed an increase in IL-1β levels from the previous year's visit were found to be twice as likely to experience disease progression the following year. Kinney et al. (2014) showed in a longitudinal study that patients that were considered as having progressive periodontal disease showed a higher level of GCF IL-1β when compared to stable periodontal patients. These results suggest that GCF IL-1β may provide a legitimate measurement of disease activity in a periodontally involved site.

IL-1 receptor antagonist (ra) is a member of the IL-1 family. The function of IL-1ra consists of binding to IL-1 receptors and antagonizing the effects of IL-1β. IL-1ra binds to receptors with nearly the same avidity as IL-1β (Arend 2002). When IL-1ra binds to the receptor,

it does not induce any cellular response, thus it antagonizes the effects of IL-1β (Dinarello 2013). IL-1ra is a 17-23 kDa protein secreted by numerous types of cells, including: immune cells such as macrophages, neutrophils, and mast cells (Roux-Lombard et al. 1989, McColl et al. 1992, Hagaman et al. 2001), epithelial cells (Perrier et al. 2002), keratinocytes (Gruaz-Chatellard et al. 1991), hepatocytes (Molnar et al. 2004), fibroblasts (Palmer et al. 2004), and adipocytes (Juge-Aubry et al. 2004). In order for IL-1ra to have its antagonist effect against IL-1β, it must be present in at least a 100-fold amount compared to IL-1β in vitro (Arend et al. 1990). Because IL-1ra does not cause signal transduction, changing the levels of production is the means by which the function of IL-1ra can be regulated. This is controlled by regulatory molecules (Perrier et al. 2006).

In periodontal disease, IL-1ra plays an anti-inflammatory role by antagonizing the effects of IL-1β. However, an increase in secretion of IL-1ra does not seem sufficient to overcome the release of IL-1β in many cases. Gilowski et al. (2014) found when comparing levels of IL-1β and IL-1ra in control and periodontitis groups that the periodontitis group showed nearly 300-times more moles/sample of IL-1ra when compared to IL-1β, suggesting that an increased amount of IL-1ra was not enough to control the effects of IL-1β in periodontitis. In control patients, IL-1ra showed nearly 800-times more moles/sample when compared to IL-1β. Holmlund et al. (2004) also reported that IL-1ra levels were higher in diseased sites when compared to non-diseased sites. On the other hand, Ishihara et al. (1997) showed no correlation between IL-1ra levels and alveolar bone loss in diseased sites.

Clinically, levels of IL-1ra do not seem to respond in a significant way to periodontal therapy. Toker et al. (2008) found that following SRP, IL-1ra levels did not significantly change in diseased sites. Yoshinari et al. (2004) also found no significant change in IL-1ra levels following

SRP. In regards to surgical therapy, Holmland et al. (2004) showed a non-significant decrease in IL-1ra levels following treatment of osseous defects caused by periodontal disease.

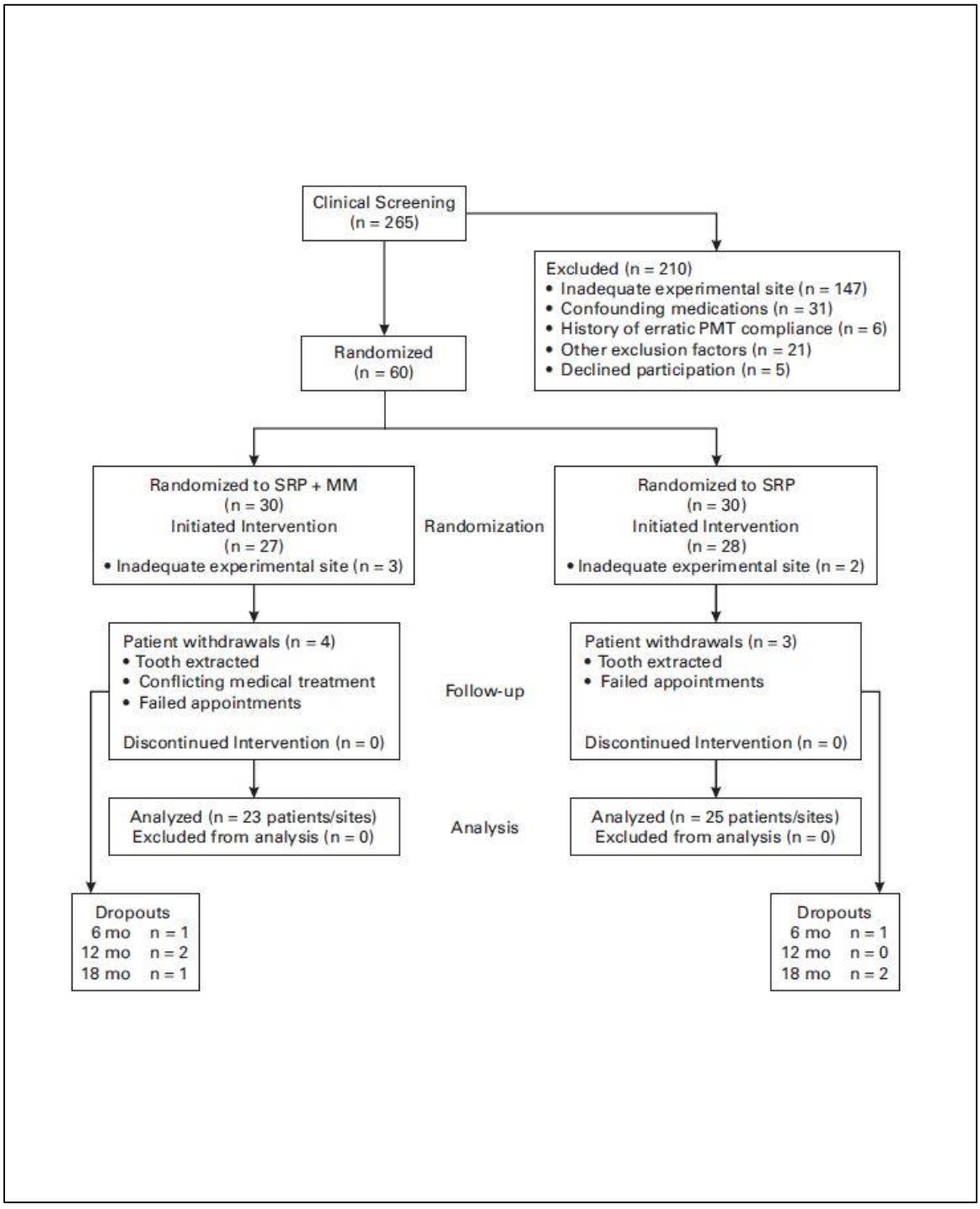
Patients experiencing periodontal disease may not have the proper levels of IL-1β and IL-1ra present in the periodontal tissues (Gilowski et al. 2014). The interaction of IL-1β and IL-1ra plays an important role in the body's inflammatory responses. When the ratio between IL-1β and IL-1ra is imbalanced, a state of disease is encountered. Periodontal disease is not alone in showing an imbalance between the two cytokines. Others include kidney disease, liver disease, arthritis, graft-versus-host disease, inflammatory bowel disease, leukemia, cancer, central nervous system disease, arterial disease, and diabetes (Arend 2002). Better understanding of the ratio of IL-1β and IL-1ra and how to possibly alter it may be helpful in the treatment of periodontal disease.

CHAPTER 5: MATERIALS AND METHODS

Patient Population and Study Design

Patients regularly attending the UNMC College of Dentistry for PMT were identified through review of their dental records by faculty (RR and AK). The inclusion criteria for the study included: subjects between the ages of 40-85 years, a periodontal diagnosis of moderate-severe chronic periodontitis, history of regular PMT at the College of Dentistry (≥2/year), at least one ≥5 mm posterior interproximal pocket with a history of BOP, and no systemic diseases (e.g. rheumatoid arthritis, osteoporosis) or medications (e.g. chronic nonsteroidal anti-inflammatory drugs, steroids, bisphosphonates, calcitonin, methotrexate, antibiotics) with significant impact on periodontal inflammation or bone turnover. Individuals meeting the inclusion criteria were invited to provide informed consent (UNMC IRB #314-12), stratified by gender and smoking status, and randomly assigned to test (SRP+MM) or control (SRP) groups by coin toss by a clinician not involved with clinical measurements (JH, Figure 1). She also identified the most posterior ≥5 mm interproximal site with a history of BOP as the experimental site. Standardized posterior vertical bitewing radiographs were exposed at the baseline and 24-month visits. Clinical measurements and GCF samples were obtained at each appointment by one of two calibrated clinicians (RR and AK). Saliva was sampled before treatment at the 24-month visit. Full mouth debridement and SRP of inflamed pockets were accomplished by the dental student assigned to the case. After completion of PMT, the test site (SRP+MM) received SRP and administration of MM in the experimental site by JH and the control site received SRP alone by JH. Periodontal maintenance therapy and SRP+MM or SRP at experimental site were performed at baseline, 6-month, 12-month, 18-month, and 24-month appointments. The study was registered with ClinicalTrials.gov (NCT01647282).

Figure 1: Study design flow chart



Data Collection and Treatment Protocol

During the baseline visit, the assignment of the test site was done by JH. Following the assignment, clinical data were collected by one of two trained and calibrated dentists (RR or AK). During data collection, supragingival plaque was removed from the test teeth with a dental explorer, then an absorbent paper slip (Periopaper, ProFLow, Amityville, NY) was inserted into the sulcus of the experimental site for 30 seconds to collect the GCF sample. Strips contaminated with blood were discarded. The paper strip was then placed into a sterile vial and frozen at -80^{o} C before further testing. Following GCF collection, gingival recession was measured at the test site using a UNC 15 probe (Hu-Friedy, Chicago, IL). Probing pocket depths were then measured at the same site. BOP was recorded as positive for sites that bled within 30 seconds. Full-mouth measurements and PMT were then completed by the dental student assigned to the case. At the end of the PMT appointment, JH performed SRP at test sites and placed MM into the experimental site pockets of patients assigned to the experimental group. Participants then returned to the College for 6-month, 12-month, 18-month, and 24-month appointments. At each appointment, full mouth debridement and root planing of bleeding sites ≥5 mm were provided, and at the baseline and 24-month visits radiographs were exposed. When exposing radiographs, a modified ring (XCP Rinn System) was used which allowed the rectangular radiographic cone to lock into a standardized film to source geometry. Measurements were made using digital imaging software (MiPACS Dental Enterprise Solution, Medicor Imaging). The measurement was made from the CEJ of the test site to the base of the bony defect (interproximal bone loss: IBL). Measurements were repeated in 10% of samples to ensure reproducibility. Saliva collection was done using a variation of the technique described by Navazesh (1993). At the beginning of the 24-month PMT, patients consented, rinsed with water and expectorated into a sterile collecting tube for five minutes while in a seated position.

Saliva samples were then centrifuged at 2,000 RPM for 5 minutes and the supernatant was pipetted into sterile vials. Vials were then frozen at -80^{o} C before further testing. At the conclusion of the study, participants returned to their normal PMT protocols.

Analysis of GCF and Salivary Samples

GCF samples from test sites were analyzed for both IL-1β and IL-1ra using standard ELISA techniques. ELISA kits utilized the quantitative sandwich technique (R&D Systems, Human IL-1β/IL-1F2 Quantikine® ELISA, R&D Systems, Minneapolis, MN; Human IL-1ra/IL-1F3 Quantikine® ELISA, R&D Systems, Minneapolis, MN). All assay procedures were completed by individuals (JJ, MS, and LE) without knowledge of treatment group allocation. The ELISA tests were performed according the manufacturer's instructions and protocol. GCF samples were allowed to thaw at room temperature. Sample strips were placed in 1 ml of phosphate buffer saline and gently agitated for 1 hour during the thawing process.

<u>IL-1β:</u>

Two hundred µl of the standard or GCF sample were pipetted into each well. Wells were pre-coated with monoclonal antibody specific for IL-1β. Samples and standards then were covered with a plate sealer and allowed to incubate for 2 hours at room temperature. All wells were then aspirated and washed a total of 3 times. Following the wash, 200 µl of polyclonal antibody specific for human IL-1β conjugated to horseradish peroxidase with preservatives were added to each well for binding to a second epitope. The samples were again covered and incubated for 2 hours at room temperature. Aspiration and washing were again done 3 times. Two hundred µl of chromogen solution stabilized with hydrogen peroxide were added to each well. The samples then were incubated for 20 minutes protected from light. Fifty µl of stop solution were added to each well and gently agitated until uniform color was obtained. The plate was then read at a 450 nm wavelength and corrected for optical imperfections using 570 nm wavelength readings subtracted from the 450 readings. All readings were done 30 minutes after the addition of the stop solution.

Standard calibration curves were generated using computer software. The minimum detectable concentration for the ELISA was 1 pg/ml and the maximum detectable concentration was 262 pg/ml. All samples were analyzed separately and in duplicate. The standard curve was used to estimate the concentration of each sample. If a sample tested at a level lower than the minimum detectable concentration it was reported as 1 pg/ml (n=18). Cytokine levels higher than the maximum detectable level were tested with a 1:10 dilution. The average of each sample's duplicate was used to determine the total IL-1β, and total IL-1β was calculated after adjusting for dilutions.

IL-1ra:

IL-1ra is typically found in large quantities in GCF and saliva. Therefore, dilutions were used to prior to testing samples on the ELISA. GCF samples were diluted at 1:10 and 1:100, while salivary samples were diluted at 1:100 and 1:1000. One-hundred µl of each sample or standard were pipetted into wells pre-coated with monoclonal antibody specific for IL-1ra. The plate was then incubated for 2 hours at room temperature. Following incubation, the wells were aspirated and washed 4 times. Two-hundred µl of polyclonal antibody against IL-1ra conjugated to horseradish peroxidase with preservatives were added to each well for binding to a second epitope. The plate was again allowed to incubate for 2 hours at room temperature. Aspiration and washing were again performed 4 times. Two-hundred µl of chromogen solution stabilized with hydrogen peroxide were added to each well and allowed to incubate for 30 minutes at room temperature while protected from light. Fifty µl of stop solution were then added to each well. Samples and standards were gently agitated until a uniform yellow color was achieved. The plate then was read at a 450 nm wavelength and optical imperfections were corrected for by subtracting the 570 nm readings to obtain the optical density of each well.

Standard calibration curves were generated using computer software. The minimum detectable concentration for the ELISA was less than 1 pg/ml and the maximum detectable concentration was 2100 pg/ml. All samples were analyzed separately and in duplicate. The standard curve was used to estimate the concentration of each sample. No samples tested at a level lower than the minimum detectable concentration. Cytokine levels higher than the maximum detectable level were measured at the 1:1000 dilution. The average of each sample's duplicate was used to determine the total IL-1ra. The levels measured in the 1:10 dilution in GCF and the 1:100 dilution in saliva were used unless the reading was over the maximum detectable level.

IL-1β/IL-1ra ratio:

IL-1β levels were multiplied by a factor of 1000 to produce ng/ml and then divided by IL-1ra levels. This created an IL-1β/IL-1ra ratio as a whole number which could be compared between subjects. This ratio has been used in the medical literature (Shingu et al. 1995, Lekovich et al. 2015).

Statistical Analyses

A power analysis was performed to optimize the power of detecting bone loss at 24 months. Based on the data, it was assumed that the standard deviation of the change in average bone loss at 24 months was 0.57 mm. The significance level was set at 0.05/2=0.025 based on Bonferroni method to adjust for two tests conducted under the two treatments separately. The current study (with either 23 or 25 subjects per group) provided 82.4% power to detect a difference of 0.4 mm in average bone loss at 24 months after treatment in each group at a two-sided significance level of 0.025 via one-sample t test.

The continuous data (bone loss, PD, CAL, GCF total IL-1β, GCF IL-1β/IL-1ra ratio) from baseline to 24 months were compared between groups using two-sample t-test or Wilcoxon rank sum test when the data was normally distributed. Categorical data (BOP and % plaque) were compared between groups using a chi-square test.

The Wilcoxon rank sum test was used to compare the change in radiographic bone loss and other clinical measurements from baseline to 24 months between the SRP+MM and SRP groups. The Fisher exact test was used to compare the relative frequency of having clinically-significant bone loss (≥1 mm) between treatment groups.

Additionally, the linear mixed effects model, including visit time, treatment, and their interaction as covariates and AR(1) correlation structure was fit to evaluate the effects of treatment on changes in the continuous outcome over time. The total IL-1β values were log-transformed to make the data normally distributed. The linear effects model was considered to account for the correlation between repeated measurements from the same patients. The generalized linear mixed effects model was used to estimate the difference in treatment effects on the change in categorical outcome over time. Stratified analysis by treatment group was used to evaluate the change of clinical outcome over time for each treatment group.

Saliva total IL-1β and saliva IL-1β/IL-1ra ratios were only available at 24 month appointments. These values were log transformed and compared using two-sample t test between groups.

Spearman correlation was calculated between the GCF total IL-1β value and saliva total IL-1β at baseline and 24 months. Spearman correlation also was calculated between each of the total IL-1β measurements at baseline or 24 months and the changes in the clinical outcome values at 24 months.

CHAPTER 6: RESULTS

Patient Characteristics

Following screening of the dental school records, 60 eligible patients were invited to participate and consented to participate in the study. Of the 60 participants randomized in the study, intervention was initiated on 55 subjects due to PD < 5 mm at baseline (Figure 1) and 48 completed the 24-month PMT (13% dropout rate).

All patients were asked to report any symptoms or problems experienced during the study. No complications were reported or noted throughout the study.

Baseline characteristics of patients initiating intervention are displayed in Table 1. There were no significant differences between groups.

Table 1: Patient characteristics across treatment groups (mean ± standard deviation)

Variables	SRP+MM (n=27)	SRP (n=28)
Age (years)	67.33 (10.52)	66.75 (12.07)
Male/Female	22 (81.5%)	16 (57.1%)
Current Smokers	8 (29.6%)	4 (14.3%)

Inflammatory Biomarker Outcomes

The mean baseline and 24-month measurements of inflammatory biomarkers are presented in Figures 2 and 3. No differences were noted at baseline or 24 months between groups.

Figure 2: Levels of IL-1β at baseline and 24 months (mean ± standard deviation). GCF=gingival crevicular fluid, SRP=scaling and root planing, M=minocycline.

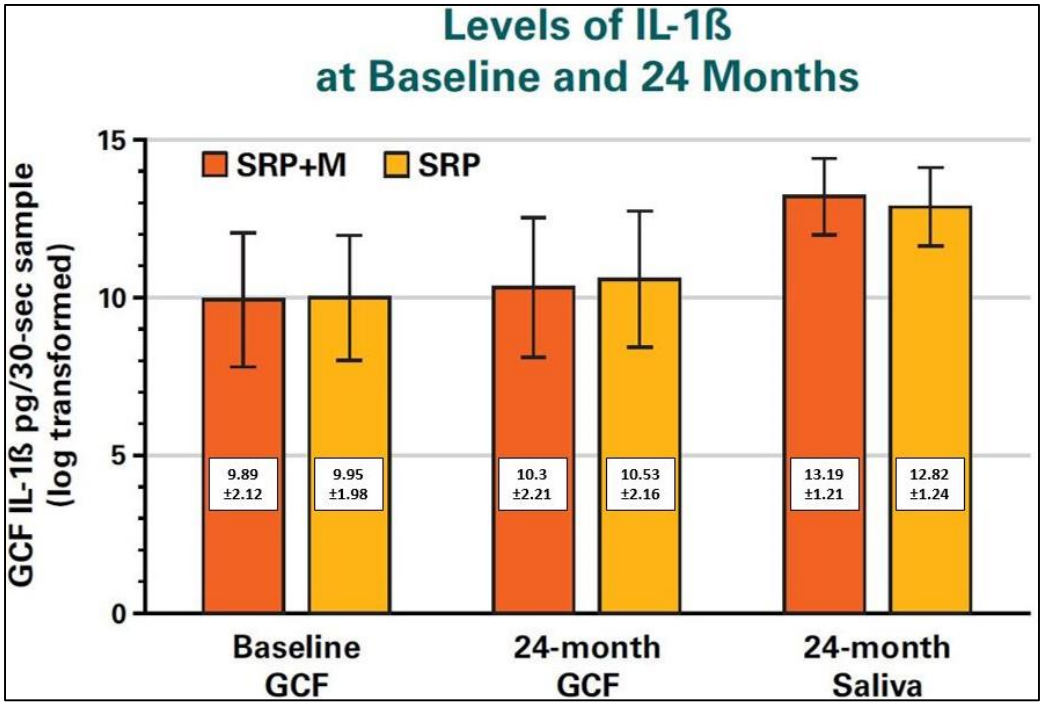
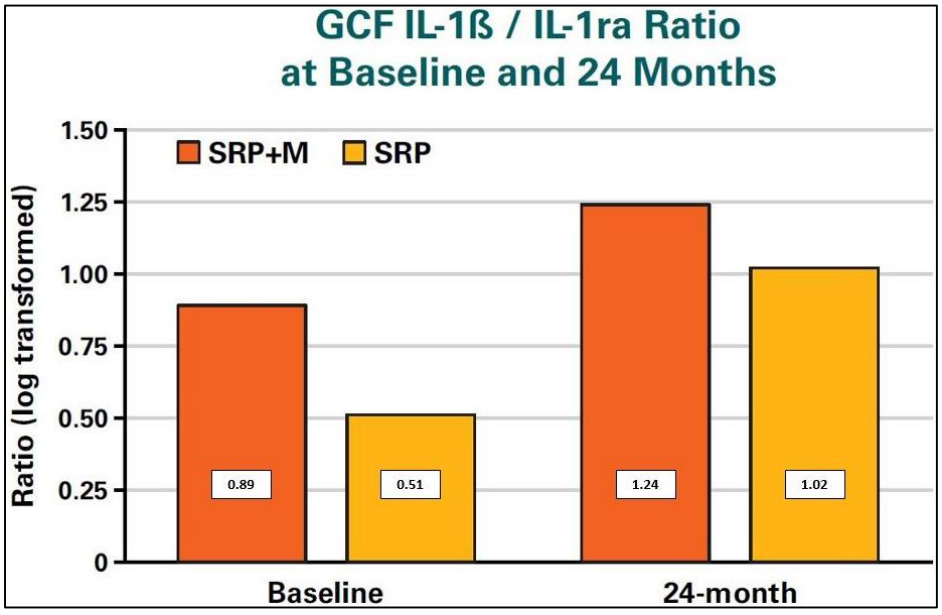


Figure 3: Mean GCF IL-1β/IL-1ra ratios at baseline and 24 months. GCF=gingival crevicular fluid.



Clinical Outcomes

Baseline and 24-month results for PD, CAL, BOP, and IBL are reported in Figures 4-6. Both SRP+MM and SRP alone significantly reduced PD, CAL, and BOP from baseline to 24 months, but there were no differences between groups at experimental sites. Both groups had stable interproximal bone heights over 24 months of 6-month PMT at experimental sites. Only one site in each group lost 0.5 mm and no sites lost ≥ 1 mm.

Figure 4: Clinical measurements at baseline and 24 months (mean ± standard deviation). CAL=clinical attachment level, SRP=scaling and root planing, M=minocycline

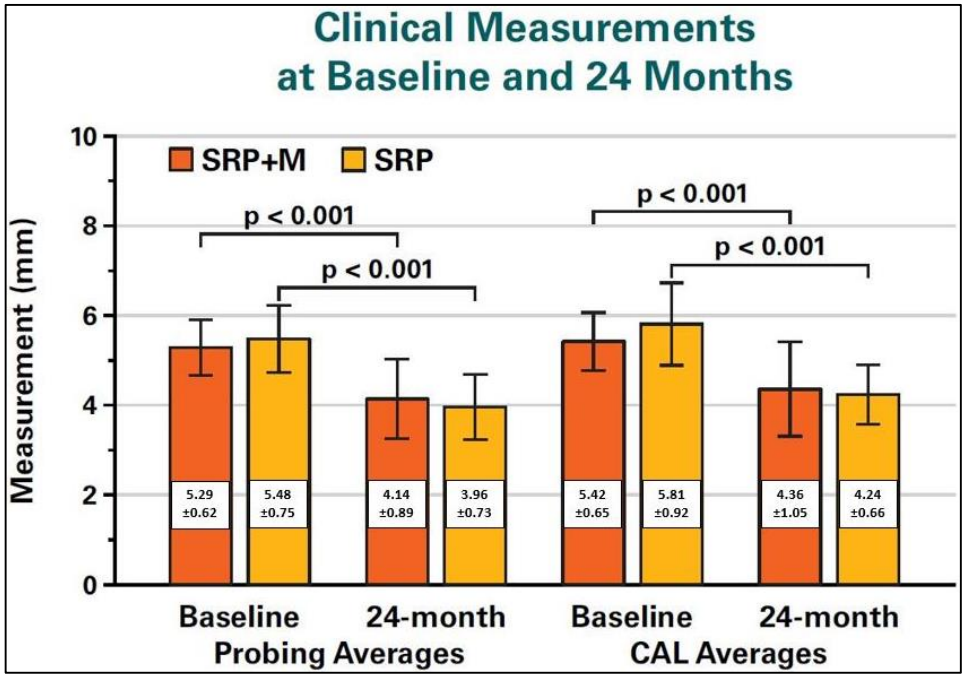


Figure 5: Percent bleeding on probing at baseline and 24 months. SRP=scaling and root planing, M=minocycline.

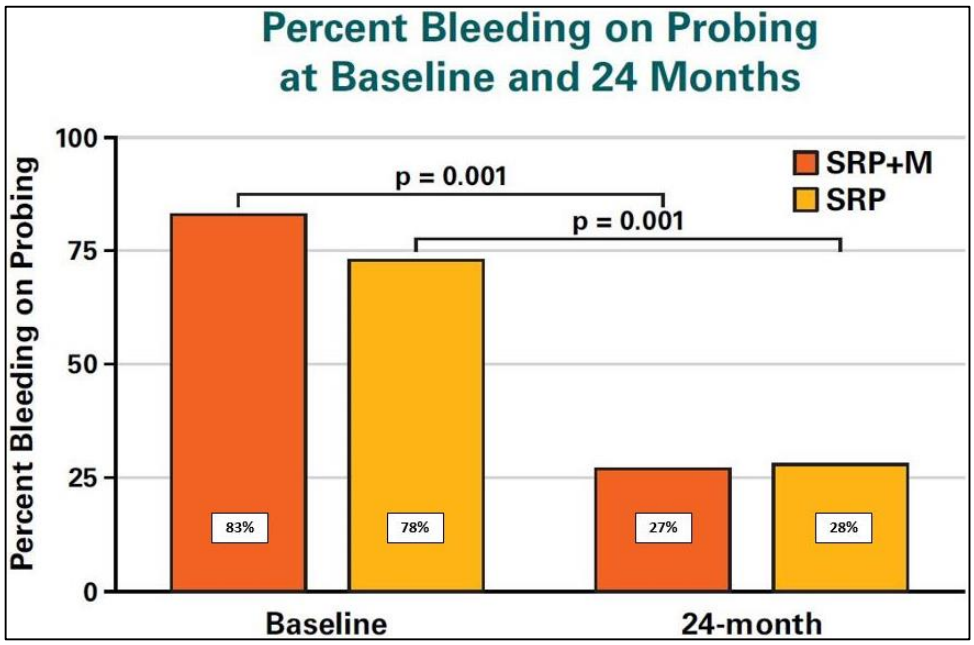
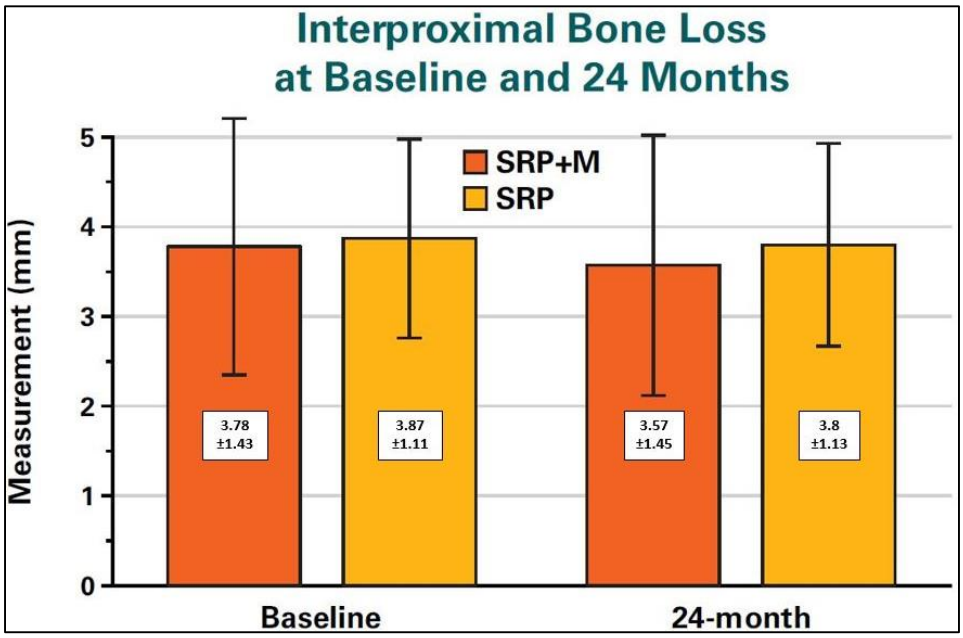


Figure 6: Interproximal bone loss at baseline and 24 months (mean ± standard deviation). SRP=scaling and root planing, M=minocycline.



A significant Spearman correlation was found between GCF total IL-1β at baseline and change in bone loss at 24 months ($r=0.34$, $p=0.017$). A concurrent correlation was found between salivary total IL-1β at 24-months and change in PD at 24 months ($r=0.34$, $p=0.031$), and a trend toward a change in CAL ($r=0.31$, $p=0.055$).

CHAPTER 7: DISCUSSION

The aim of this study was to determine the effect of MM when used in conjunction with SRP in patients receiving regular PMT compared to SRP alone during PMT. Patients included in the study were patients who regularly attended PMT appointments at the University of Nebraska Medical Center College of Dentistry. All patients had received periodontal treatment prior to enrolling in the study. Each patient had at least one posterior pocket ≥5 mm with a history of BOP. Patients received PMT at 6 month intervals.

The primary outcome measure in this study was change in interproximal bone height. Neither group demonstrated a significant change from baseline to 24 months even though the study was powered to detect changes of 0.4 mm mean bone loss. In fact, only one site in each group lost between 0.5 and 1.0 mm IBL during the 24 months. Also, there was no difference between groups at baseline and 24 month appointments in IBL. Mean changes of <0.2 mm in the current study are far short of a 1 mm change in bone height needed to achieve reproducibility in bone height changes when investigating a series of radiographs (Hausmann et al. 1997). Previous studies have indicated that bone height changes during regular PMT average <1 mm over a 14 year period (Lindhe & Nyman 1984) and patients not receiving regular PMT experienced a significant amount of alveolar bone loss (Becker et al. 1984a). Payne et al (2013) reported that in post-menopausal women receiving sub-antimicrobial doses of doxycycline or placebo and regular PMT, alveolar bone height remained stable in both groups. The test group and the control experienced no significant loss or gain in alveolar bone height during the 2-year study. At 1 year, 94% of sites showed no change in alveolar bone height and at 2 years 90% of sites showed no change in alveolar bone height. In the current study, 96% of subjects showed no change in alveolar bone height during the 24 months. Results in the present study indicate a

population with stable alveolar bone height at experimental sites, presumably supported by repeated SRP.

When evaluating the baseline and 24 month clinical and inflammatory biomarker measurements, no significant differences were found between the two groups. However, both groups experienced a statistically significant decrease in PD, CAL, and BOP. No changes were found from baseline to 24 months in IL-1β levels in either group. Meinberg et al. (2002) reported an improvement in PD of 0.9 ± 0.1 mm in the SRP+MM group at one year with a 0.4 ± 0.1 mm in the SRP group. CAL levels were not reported. However, Meinberg et al. only followed patients for 1 year. Meinberg et al. also administered 4 doses of MM over a 6 month period with only one session of SRP in contrast to the current study which administered the 4 doses of MM and the 4 sessions of SRP every 6 months for 2 years. The current study layout may better approximate a clinically feasible situation. In this study, it was shown that at 24 months a PD reduction of 1.1 ± 0.9 mm in the SRP+MM group and 1.5 ± 0.6 mm in the SRP group was achieved. This reduction appears to coincide with Meinberg et al. SRP+MM data at one year, but 6-month SRP promoted more 2-year PD reduction than the one episode of SRP caused at one year. In fact, PD numerically reduced further (not statistically significant) at the 24-month PMT following the initial improvements at 6 months when evaluating data from the current study previously published (Killeen et al. 2016). Previous reports (Lindhe et al. 1982, Cobb 1996) also showed that at 1 year post initial therapy a 1 mm decrease in PD can be expected following acceptable SRP without adjunctive therapy. The reductions in PD in both SRP+MM and SRP groups were significant, however, there was no difference between the two groups at 24 months. This suggests that repeated SRP alone during PMT can result in stable PD over a 2-year period.

When considering CAL, the current study showed a significant gain in CAL in both groups. The SRP+MM group showed a gain of 1.0 ± 1.0 mm and the SRP group showed a gain of 1.6 ± 0.8 mm at the end of 24 months. Both groups showed a significant improvement in CAL gain, but again, the difference between groups was not statistically significant. This improvement in CAL falls in line with previous data regarding post-SRP response to therapy (Cobb, 1996). Previous studies evaluating PMT patients showed results ranging from no change in CAL during a 14-year therapy period (Lindhe & Nyman 1984) to a slight gain (0.3-0.4 mm) in CAL over a 30-year PMT period (Axelsson et al. 2004).

The current study showed that both the SRP+MM and SRP groups demonstrated a significant decrease in the percentage of BOP. The SRP+MM group showed a decrease of 55% and the SRP group showed a decrease of 48%. There was no statistically significant differences in BOP between the groups at baseline or 24 months. This reduction in BOP following therapy is consistent with previous findings (Cobb 1996). The presence of BOP is not a reliable predictor of disease activity, but the reduction and elimination of BOP may be used as a criterion for stability (Chavez et al. 1990, Lang et al. 1990). Using BOP as a sign of periodontal stability, this study would reinforce the idea that repeated SRP can lead to a reduction in BOP and thus increase periodontal stability regardless of the addition of MM in PMT. This finding is in agreement with previous studies in regards to BOP and PMT. Miyamoto et al. (2006) showed that patients who were compliant with PMT showed a greater decrease in BOP levels when compared to poorly compliant patients. Novaes et al. (1996) found that patients who were compliant with PMT were able to maintain the amount of BOP between appointments whereas non-compliant patients saw an increase in the amount of BOP between appointments.

Periodontal disease and severity of disease have been associated with GCF IL-1β levels (Ishihara et al. 1997, Yoshinaria et al. 2004, Toker et al. 2008). IL-1β is a pro-inflammatory cytokine released by various cells in the periodontium. IL-1ra is an anti-inflammatory cytokine that has an antagonistic effect on IL-1β. In the current study, total IL-1β/30 second sample was measured as well as the IL-1β/IL-1ra ratio. At baseline, there was no significant difference between total IL-1β in the SRP+MM group (9.9 ± 2.1 pg, log transformed) and SRP group (10.0 ± 2.0 pg, log transformed). At 24 months, no significant difference was noted between the SRP+MM group (10.3 ± 2.2 pg, log transformed) and the SRP group (10.5 ± 2.2 pg, log transformed). When looking at the IL-1β/IL-1ra ratio, there was no significant difference between groups at baseline (SRP+MM 0.89 ± 1.2 log scale, SRP 0.51 ± 1.7 log scale) or 24 months (SRP+MM 1.2 ± 2.4, SRP 1.0 ± 2.0). Neither group experienced a significant change from baseline to 24 months in the IL-1β/IL-1ra ratio. Many studies report that SRP produces a reduction in GCF IL-1β at various time periods (Engebretson et al. 2002, Toker et al. 2008). These studies followed patients for 6 weeks and 24 weeks, respectively. In addition, these studies were following patients after initial SRP rather than starting with patients in ongoing PMT. These findings suggest that baseline IL-1β levels were already lowered by previous PMT and that BOP may be a simpler and more sensitive measure of local inflammation.

Salivary levels of IL-1β have been shown to reflect periodontal disease severity (Kinney et al. 2011, Sexton et al. 2011, Rathnayake et al. 2013). In the current study, total IL-1β was measured at the 24-month PMT appointment. Results were similar between the SRP+MM group (13.2 ± 1.2 pg, log transformed) and SRP group (12.8 ± 1.2 pg, log transformed). Much like GCF IL-1β, evidence shows that periodontal therapy may reduce the amount of IL-1β in saliva (Sexton et al. 2011). These findings again suggest that salivary IL-1β at 24 months may have already been lowered by previous PMT.

During the current study several attempts were made to avoid any bias. The manufacturer of MM did not support the study in anyway. Clinical and inflammatory biomarker data were measured and collected by different clinicians masked to experimental groups (RR and AK) than the clinician providing randomization and therapy (JH). ELISA testing was carried out by individuals not involved with collection or providing of therapy (JJ, MS, or LE). One potential area of bias may arise in the fact that the clinician providing therapy (JH) did both the SRP and the application of MM to the test sites. Due to the knowledge of what sites received MM and those that did not, the clinician may have provided different levels of SRP intensity in each group. This does not appear to be the case due to the fact that both test and control sites experienced a similar improvement in CAL and PD.

Current smokers were included in both the SRP+MM group (n=8) and SRP group (n=4). Smoking has been shown to affect the severity of periodontal disease and the patient's response to therapy. Cigarette smoking has been shown to be associated with a 2- to 8-fold increased risk for CAL loss and BL (Johnson & Guthmiller 2007). Bergstrom (2004) found that over a 10-year period smokers lost more periodontal bone height (0.74 ± 0.59 mm) than non-smokers (0.27 ± 0.29 mm). Labriola et al. (2005) found that PD ≥ 5 mm were reduced more in non-smokers when compared to smokers during SRP by an average of 0.433 mm. Previous data published from this study found no difference between the clinical outcomes of smokers and non-smokers at 1 year (Killeen et al. 2016).

This study does present with several limitations. Patients selected for this study were receiving regular PMT at the College of Dentistry. This regular care suggests that patients were likely stable periodontally. In order to avoid this limitation, future studies may consider enrolling patients that present for initial SRP and following them through a period of PMT. Several

patients also dropped out of the study for various reasons. These drop outs may have affected the power of the study. However, the statistical analysis showed that the study had sufficient power with the amount of patients that were retained through the whole study. Based on our data, it was assumed the standard deviation of the change in average bone loss at 24 months was 0.6 mm. The significance level was set to be 0.05/2=0.025 based on Bonferroni method to adjust for two tests conducted under the two treatments separately. Our study provided 82.4% power to detect a difference of 0.4 mm in average bone loss at 24 months after treatment in each group at a two-sided significance level of 0.025 via one-sample t test.

CHAPTER 8: CONCLUSIONS

Scaling and root planing of inflamed moderate periodontal pockets, with or without the addition of MM at 6 month intervals, produced a long-term improvement in BOP, PD, CAL, and stable interproximal bone heights after 24 months of 6-month PMT. SRP with or without MM did not demonstrate a significant improvement in total IL-1β or IL-1β/IL-1ra ratios for patients with a history of PMT compliance.

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Appendix A: Raw Clinical Data

Patient	Group	Age	Gender	Smoking	Baseline IL1-B	6-mo IL1-B	12-mo IL1-B
N1	1	77	1	2	34366	16398	433
N2	2	75	1	2	124981	19241.5	156716
N3	2	71	2	2	126209.5	132026.5	225227
N4	2	66	2	1	61705.5	1000	8319
N5	1	67	1	2	57310	78122.5	115997
N6	1	48	1	2	21503.5	1000	45353
N7	2	59	2	3	80772	1000	24477
N8	1	75	1	2	80708	27644	79996.5
N9	1	57	1	3	172163	175524.5	251209
N10	2	52	2	2	48585	75924.5	55242
N11	1	54	2	1	91566	39601	
N12	2	40	1	1	1000		1000
N14	2	72	1	2	24154	17367.5	61123.5
N15	2	61	1	1	1000	2178.5	433.5
N17	2	69	1	3	330525	34176.5	196984.5
N19	2	75	2	3	42653	1000	1000
N20	1	60	1	1	1897300	215330	73289.5
N21	2	82	2	3	138618	1000	24489
N22	1	60	1	1	1000	1000	87215
N23	1	74	1	1	1000	1000	1000
N24	2	57	1	2	1000	60756	38778
N25	1	69	1	1	2692.5	47436	84672
N26	2	73	2	2	9897.5	4448.5	64328.5
N27	2	45	1	2	39444	26305.5	50039.5
N28	1	51	2	1	1000	1000	1000
N29	1	69	1	2	1000	1000	1000
N30	2	84	1	2	4206	78254	34176.5
N31	1	69	2	2	12766	25916	7677.5
N33	1	79	1	2	1000	1268	7347
N34	2	71	1	2	13162.5	7677.5	1000
N35	2	62	2	1	1000	13625	1000
N36	1	67	1	3	168452	31202.5	21752.5
N37	2	74	1	2	262000	110697.5	79970
N38	1	60	1	3	1000	1000	1000
N39	2	76	2	2	1000	1000	1000

N40	1	73	1	2	25718	70652.5	187086.5
N41	1	59	2	3	21752.5	58494	16730.5
N42	2	50	1	2	89551.5	222968	1000
N43	1	69	2	2	67018.5	1000	60410
N44	2	81	1	2	62185	7782	3217
N45	2	46	2	3	45051	17474	147791
N46	1	56	1	1	15098	1984	20163
N47	1	83	1	2	153932	88011	76817
N48	2	70	1	2	1000	38860	11722
N49	2	65	2	3	3530	17162	7594
N50	1	85	1	3	98578	157496	38548
N51	1	77	1	2	67562	36234	23790
N52	2	75	1	2	262500	119464	62873
N53	2	83	1	3	53993	152369	61309
N54	1	66	1	1	34233	1108	3922
N55	2	76	1	3	36734	29355	6844

18-mo IL1-B	24-mo IL1-B	Saliva 24-mo	Baseline IL1-ra	6-mo IL1-ra
40485.5	140898	74205	18160.1	15158.765
3105	86781.5	672870	30204.55	16679.94
87407.5	262500	403110	22945.6	32301.55
1000			18375.15	1676.335
15148	163107.5		8374	22300.4
6232.5	16868.5	1119345	18697.8	1003.815
1000	301	72040	20687.25	1660.32
168112	179217	473795	31925.2	12933.04
262500	262500	98725	36925.8	42087.7
63477	20153		14557.55	16762.1
13271.5	127603	1215275	13858.5	21977.75
32821.5	262500		447.85	
95877	254200	532220	11438.9	22246.6
1000	2674.5	14335	1276.025	11008.7
259130	178260.5	129020	35689.1	20902.4
66471	1301	341080	4407.525	4230.115
227921	233865.5	876270	92953.95	24988.9
96629.5	150132	974365	65854	3148.755
1079.5	2964.5	95120	56.74	833.97
11228.5	58279	228555	251.05	5894.39
17246	60309	33815	504.495	3106.51
63934	1000	159315	1602.75	26279.35
40952.5	48129.5	276165	9163.815	9476.395
47694	61179	260290	1340.86	11157.57
1000	17536		580.53	893.105
1000	1000	97280	605.87	225.705
7893.5	1000	1214555	1340.86	10667.575
66834	130558	579100	2992.095	32247.8
37256.5	150349.5	484615	2301.57	12050.52
34282.5	1000	268225	18767.435	16350.605
10648.5	53421.5	551695	2558.95	16256.44
22756	85755	1803120	5451.61	2841.59
5501.5	199516.5	810635	130792.4	55793.4
2809	1000		1083.74	10506.26
39514.5	7202.5	1181290	8779.945	2916.765
144529	262500	791160	65433.4	10443.485

150481			25451.6	43237.3
136451	262500	1572310	14787.51	24294.8
214255	142545	3163000	19985.27	3444.075
56237.5	116468.5	1200130	1162.58	2518.99
109807.5	112500	289145	51592.3	14154.93
158134			3230.45	1541.57
119586.5	1000	1047935	192321.1	198063
75653			6096.21	2751.705
	145379	1324185	167578.7	58900.2
256063	240048	3763750	124879.9	161941.2
27185	9895	1583130	163402.8	184386
262500	262500	439175	210000	210000
1000			210000	179271.3
221199.5	1000	584870	10923.47	89175.7
			114544.5	115170.9

12-mo IL1-ra	18-mo IL1-ra	24-mo IL1-ra	Saliva 24-mo	Baseline Ratio
11523.95	7863.335	3186.265	434803	1.89239046
44023.4	1599.635	6595.295	2058341	4.137820295
81124.6	51382.9	2811.55	624669.5	5.500379158
18313.205	885.8			3.358095036
33699.6	522.76	5798.8		6.843802245
12352.95	3046.085	1191.82	1703990.5	1.150055087
13253.29	1535.915	280.625	292019.5	3.90443389
26601.95	1382.985	1841.77	2100000	2.52803428
45260.1	465.41	465.41	1237769	4.662404064
31172.4	2236.835	18829.585		3.337443457
	2134.88	3817.095	2100000	6.607208572
8625.71	363.46	643.825		2.232890477
43001.8	32710.4	32656.4	1573489.5	2.111566672
21977.75	20985.65	22390.4	456809	0.783683705
4737	37465.2	24065.4	583216.5	9.26123102
5936.635	17041.3	8890.01	2100000	9.677313231
1332.41	35303.95	28550	1546878	20.4111821
2346.185	24011.4	76205.7	2100000	2.104929086
14283.37	5042.95	9496.115	1394882	17.62425097
487.6	10750.405	25416.2	1537154	3.983270265
4086.495	13865.095	19345.265	427638.5	1.9821802
665.005	17797.75	13301.085	1039714.5	1.679925129
7406.605	18789.675	13475.25	937360.5	1.080063271
20653.25	23957.3	31900	2075741	29.41694137
10	631.88	16987.3		1.722563864
22.951	5556.455	16014.75	2100000	1.650519088
4018.91	7602.045	8687.98	2100000	3.136792805
3343.635	20445.3	14496.45	2100000	4.26657576
16733.53	18716.3	28928.2	1754656	0.434486025
7988.98	5783.74	3283.575	698876	0.70134784
3714.005	11196.565	22984.75	1457829.5	0.390785283
19037.37	13570.465	33088.65	2100000	30.89949575
47599.4	19028.185	30456.75	1845751	2.003174496
5182.94	6838.19	5793.56		0.922730544
1466.66	17766.595	20769.435	2100000	0.11389593
39622.3	20865.865	43571.7	2100000	0.393040863

23282.6	42114.5			0.854661397
12100.74	16143.41	46902.45	2100000	6.055887705
22125.8	37326.45	27334.15	2100000	3.353394775
2425.9	13459.52	30248.55	2100000	53.48879217
13915.57	17830.88	15572.875	2100000	0.8732117
18623.14	12093.47			4.673652278
79779.9	34620.2	7264.075	1645649.5	0.800390597
120912.8	8951.55			0.164036344
57229.8		35557	2100000	0.02106473
71428	19839.85	30456.75	2100000	0.789382439
19175.01	17927.305	5865.885	2100000	0.413469047
72158.8	37534.65	48151.55	1798156.5	1.25
17020.7	2209.69			0.257109524
151083.8	45028.95	1735.59	2100000	3.133894266
4480.48				0.320696323

6 month Ratio	12 month Ratio	18 month Ratio	24 month Ratio	Saliva Ratio
1.081750393	0.037573922	5.148642402	44.22042737	0.170663496
1.153571296	3.559834088	1.941067806	13.15809225	0.326899187
4.0873116	2.776309529	1.70110095	93.36486991	0.645317244
0.596539475	0.454262375	1.128923007		
3.503188284	3.442088333	28.9769684	28.1278023	
0.996199499	3.671430711	2.04606897	14.15356346	0.656896268
0.602293534	1.846862175	0.651077696	1.072605791	0.246695854
2.137471159	3.007166768	121.557356	97.30693843	0.225616667
4.170446472	5.55034125	564.0188221	564.0188221	0.07976044
4.529533889	1.772144589	28.37804308	1.070283811	
1.801867798		6.216508656	33.42934876	0.578702381
	0.115932486	90.30292192	407.719489	
0.780681093	1.421417243	2.931086138	7.784079078	0.33824185
0.197888942	0.019724494	0.04765161	0.119448514	0.03138073
1.635051477	41.58423053	6.916551893	7.407335843	0.22122145
0.236400192	0.168445592	3.900582702	0.146344042	0.162419048
8.61702596	55.00521611	6.455963143	8.191436077	0.566476477
0.317585839	10.43779583	4.024317616	1.970088852	0.463983333
1.1990839	6.106052003	0.214061214	0.312180297	0.068192148
0.169652839	2.050861362	1.044472278	2.292986363	0.148687119
19.55763864	9.489305627	1.243842902	3.117507049	0.079073797
1.805067477	127.3253585	3.59225183	0.075181837	0.153229564
0.469429567	8.685288334	2.179521466	3.571696258	0.294619839
2.357637012	2.42283902	1.990791951	1.917836991	0.125396184
1.119689174	100	1.582578971	1.032300601	
4.430562017	43.57108623	0.179970863	0.062442436	0.04632381
7.33568782	8.503922705	1.038339026	0.115101554	0.578359524
0.803651722	2.296153737	3.268917551	9.006204967	0.275761905
0.105223675	0.439058585	1.990591089	5.197333398	0.276188039
0.46955449	0.125172425	5.927393002	0.304546112	0.383794836
0.838129381	0.269251118	0.951050612	2.324214969	0.378435887
10.98064816	1.142621066	1.676876953	2.591674184	0.858628571
1.984060839	1.680063194	0.289123739	6.550813859	0.439189793
0.095181349	0.192940686	0.410781215	0.172605445	
0.342845584	0.681821281	2.224089647	0.346783627	0.562519048
6.765222529	4.721747602	6.926576013	6.024552634	0.376742857

1.352859684	0.718583835	3.573139892		
9.177601791	0.082639574	8.452427337	5.596722559	0.748719048
0.290353723	2.730296758	5.740031533	5.214905164	1.506190476
3.089333423	1.326105775	4.178269359	3.850382911	0.571490476
1.234481555	10.6205495	6.158277101	7.224099596	0.137688095
1.286999617	1.082685304	13.07598233		
0.444358613	0.962861573	3.454240588	0.137663777	0.636791127
14.12215336	0.096945898	8.451385514		
0.291374223	0.132693107		4.088618275	0.630564286
0.972550531	0.539676317	12.90649879	7.8816026	1.792261905
0.196511666	1.240677319	1.516401935	1.686872484	0.753871429
0.56887619	0.871314379	6.993537971	5.451537905	0.24423625
0.849935266	3.602025769	0.452552168		
0.012424909	0.025959103	4.912384144	0.576172944	0.278509524
0.254882093	1.527514909			

Baseline, Site, F probe	Baseline, Site, L probe	Baseline treatment site Probe	Baseline, Site, Average Probe	Baseline, Site, F rec	Baseline, Site, L rec	Baseline treatment site Rec
5	3	5	4	1	0	1
5	5	5	5	0	0	0
3	6	6	4.5	0	0	0
3	4	4	3.5	1	1	1
3	6	6	4.5	0	0	0
3	4	4	3.5	0	0	0
4	6	6	5	0	2	2
3	6	6	4.5	2	0	0
5	5	5	5	0	0	0
2	5	5	3.5	3	0	0
5	6	6	5.5	0	0	0
6	5	6	5.5	1	0	1
5	7	5	6	1	0	1
7	4	7	5.5	1	0	1
5	5	5	5	0	1	0
5	4	5	4.5	1	0	1
5	6	6	5.5	1	0	0
5	4	5	4.5	0	0	0
3	5	5	4	1	1	1
5	4	5	4.5	0	0	0
4	6	6	5	1	0	0
5	5	5	5	0	0	0
5	4	5	4.5	1	2	1
4	6	6	5	0	0	0
5	3	5	4	0	0	0
5	4	5	4.5	0	0	0
5	5	5	5	0	0	0
5	5	5	5	0	0	0
3	5	5	4	2	0	0
4	6	6	5	0	0	0
3	5	5	4	1	0	0
5	5	5	5	0	0	0
5	6	6	5.5	0	0	0
3	5	5	4	0	0	0
3	5	5	4	1	1	1
5	4	5	4.5	0	3	0

5	5	5	5	0	0	0
5	5	5	5	0	0	0
4	6	6	5	0	0	0
3	5	5	4	0	0	0
6	4	6	5	0	0	0
5	7	7	6	1	0	0
6	7	6	6.5	0	0	0
4	5	5	4.5	0	0	0
5	3	5	4	0	0	0
5	3	5	4	0	0	0
3	5	5	4	2	0	0
7	6	7	6.5	0	1	0
5	5	5	5	0	0	0
3	5	5	4	2	1	1
7	8	7	7.5	0	1	0

Baseline, Site, Average Rec	Baseline, Site, CAL	Baseline, Site, F BOP	Baseline, Site, L BOP	Baseline treatment site BOP	Baseline treatment site BOP average	Baseline, Adj, F probe
0.5	4.5	1	0	1	0.5	
0	5	0	1	1	0.5	
0	4.5	1	1	1	1	
1	4.5	0	0	0	0	
0	4.5	0	1	1	0.5	4
0	3.5	0	0	0	0	3
1	6	1	1	1	1	5
1	5.5	0	1	1	0.5	4
0	5	1	1	1	1	3
1.5	5	0	1	1	0.5	3
0	5.5	0	1	1	0.5	8
0.5	6	0	1	0	0.5	5
0.5	6.5	1	1	1	1	4
0.5	6	0	0	0	0	5
0.5	5.5	1	1	1	1	3
0.5	5	0	0	0	0	4
0.5	6	1	0	0	0.5	5
0	4.5	1	1	1	1	5
1	5	0	1	1	0.5	3
0	4.5	1	1	1	1	3
0.5	5.5	1	1	1	1	4
0	5	0	1	1	0.5	4
1.5	6	0	0	0	0	4
0	5	1	1	1	1	3
0	4	0	0	0	0	2
0	4.5	1	1	1	1	3
0	5	1	1	1	1	6
0	5	0	1	1	0.5	4
1	5	1	1	1	1	3
0	5	0	1	1	0.5	4
0.5	4.5	1	1	1	1	3
0	5	1	1	1	1	3
0	5.5	1	1	1	1	3
0	4	0	1	1	0.5	3
1	5	0	0	0	0	3
1.5	6	0	0	0	0	3

0	5	1	1	1	1	4
0	5	1	1	1	1	3
0	5	0	1	1	0.5	5
0	4	1	1	1	1	3
0	5	1	1	1	1	5
0.5	6.5	1	1	1	1	3
0	6.5	1	1	1	1	4
0	4.5	1	1	1	1	4
0	4	1	0	1	0.5	3
0	4	1	1	1	1	4
1	5	1	1	1	1	3
0.5	7	1	1	1	1	5
0	5	1	1	1	1	5
1.5	5.5	0	1	1	0.5	2
0.5	8	1	1	1	1	6

Baseline, Adj, L probe	Baseline, Adj, F rec	Baseline, Adj, L rec	Baseline, Adj, F BOP	Baseline, Adj, L BOP	6 month, Site, F probe	6 month, Site, L probe
					3	3
					3	5
					3	5
					3	4
6	2	0	1	1	4	6
3	2	0	0	0	3	4
3	0	0	0	1	4	5
6	0	0	0	1	4	6
3	1	0	1	1	6	6
5	2	0	0	0	2	5
7	0	0	1	1	6	6
6	0	0	1	1		
6	1	0	1	1	5	6
5	1	0	0	0	5	4
4	0	0	0	1	4	5
5	0	0	0	0	5	5
5	0	0	0	0	4	5
4	1	0	1	1	4	4
3	0	0	0	0	4	4
3	1	0	0	0	4	2
4	2	0	0	0	3	5
5	0	0	0	0	3	4
4	1	1	0	0	3	4
5	0	0	1	1	3	5
2	0	0	0	0	3	3
3	0	0	0	1	4	3
6	0	0	1	1	4	5
4	0	0	0	0	4	5
4	1	0	1	1	3	4
6	0	0	0	0	4	4
3	0	0	0	0	3	3
4	0	0	0	0	4	4
3	1	1	0	0	4	5
4	0	0	0	0	3	4
4	1	1	0	0	3	5
4	0	2	0	0	4	5

3	0	0	0	0	3	4
4	0	0	0	1	4	6
4	0	0	0	0	4	6
5	1	0	1	0	4	6
3	0	0	1	1	5	4
7	0	2	0	0	5	6
5	4	0	0	0	3	5
4	0	0	1	0	3	4
3	0	0	0	0	3	3
5	0	0	1	1	4	4
5	2	0	1	1	4	4
3	0	0	1	1	7	4
4	0	0	1	1	4	4
4	0	0	0	0	3	4
6	0	1	1	1	9	4

6 month treatment site Probe	6 month, site, average, probe	6 month, Site, F rec	6 month, Site, L rec	6 month treatment site Rec	6 month, treatment site, rec, average
3	3	1	1	1	1
5	4	0	0	0	0
5	4	0	0	0	0
4	3.5	0	0	0	0
6	5	1	0	0	0.5
4	3.5	0	0	0	0
5	4.5	1	0	0	0.5
4	5	0	0	0	0
6	6	0	0	0	0
5	3.5	4	0	0	2
6	6	0	0	0	0
5	5.5	0	0	0	0
5	4.5	0	0	0	0
4	4.5	1	0	1	0.5
5	5	1	1	1	1
5	4.5	0	0	0	0
4	4	1	0	1	0.5
4	4	0	0	0	0
4	3	0	0	0	0
5	4	1	0	0	0.5
4	3.5	0	0	0	0
3	3.5	2	2	2	2
5	4	0	0	0	0
3	3	0	1	0	0.5
4	3.5	1	0	1	0.5
5	4.5	1	0	0	0.5
5	4.5	0	0	0	0
4	3.5	1	0	0	0.5
4	4	0	1	1	0.5
3	3	1	0	0	0.5
4	4	0	0	0	0
5	4.5	1	0	0	0.5
4	3.5	0	0	0	0
5	4	0	0	0	0
4	4.5	0	0	0	0

4	3.5	0	0	0	0
6	5	0	0	0	0
6	5	0	0	0	0
6	5	0	0	0	0
5	4.5	0	0	0	0
6	5.5	1	0	0	0.5
3	4	1	2	1	1.5
4	3.5	0	1	1	0.5
3	3	0	0	0	0
4	4	0	0	0	0
4	4	2	0	0	1
7	5.5	0	2	0	1
4	4	0	0	0	0
4	3.5	2	0	0	1
9	6.5	0	0	0	0

6 month, CAL	6 month, Site, F BOP	6 month, Site, L BOP	6 month treatment site BOP	6 month site, BOP, average	6 month, Adj, F probe
4	1	1	1	1	2
4	1	0	0	0.5	4
4	0	1	1	0.5	3
3.5	0	0	0	0	3
5.5	1	1	1	1	3
3.5	0	0	0	0	3
5	0	1	1	0.5	4
5	0	0	0	0	5
6	1	1	1	1	6
5.5	0	1	1	0.5	2
6	0	0	0	0	2
5.5	1	0	1	0.5	5
4.5	0	0	0	0	3
5	0	1	0	0.5	3
6	0	0	1	0	5
4.5	0	0	0	0	3
4.5	0	0	0	0	4
4	0	0	0	0	4
3	1	0	1	0.5	2
4.5	0	0	0	0	4
3.5	0	0	0	0	4
5.5	0	0	0	0	2
4	0	0	0	0	3
3.5	0	0	0	0	3
4	0	0	0	0	1
5	1	1	1	1	6
4.5	0	0	0	0	3
4	0	0	0	0	3
4.5	0	0	0	0	6
3.5	0	0	0	0	3
4	0	0	0	0	3
5	1	0	0	0.5	3
3.5	0	0	0	0	3
4	0	0	0	0	3
4.5	1	0	1	0.5	3

3.5	0	0	0	0	2
5	1	1	1	1	3
5	1	1	1	1	3
5	1	0	0	0.5	3
4.5	1	1	1	1	5
6	0	1	1	0.5	3
5.5	1	1	1	1	2
4	0	0	0	0	3
3	0	0	0	0	1
4	1	1	1	1	4
5	1	0	0	0.5	4
6.5	1	1	1	1	4
4	1	1	1	1	4
4.5	0	0	0	0	3
6.5	1	1	1	1	7

6 month, Adj, L probe	6 month, Adj, F rec	6 month, Adj, L rec	6 month, Adj, F BOP	6 month, Adj, L BOP	12 month, Site, F probe
2	4	3	0	1	4
5	0	0	0	0	4
4	0	0	0	1	3
4	0	0	0	0	3
4	0	0	0	0	2
4	0	0	0	0	3
4	1	0	0	0	4
5	1	0	0	1	5
4	0	0	1	1	6
5	3	0	0	1	2
2	0	0	0	0	
					6
6	1	0	0	0	5
3	1	1	0	0	5
5	0	0	0	0	5
4	1	1	0	1	5
5	0	0	0	0	4
4	1	0	0	0	4
4	0	1	1	0	4
3	0	0	0	0	4
3	1	0	0	0	3
4	0	0	0	0	4
3	2	2	0	0	2
5	0	0	1	1	4
3	0	0	0	0	3
1	1	1	0	0	4
6	0	0	1	1	4
3	0	0	0	0	4
3	1	0	1	1	3
5	0	0	0	0	4
3	1	0	0	0	3
4	0	0	0	1	3
5	0	0	1	0	4
4	0	0	0	0	3
4	1	0	0	0	3
5	0	0	0	0	4

2	1	1	0	0	4
3	0	0	0	1	4
5	0	0	1	1	4
4	1	0	1	0	2
3	0	0	1	1	4
5	0	1	0	1	6
4	2	2	1	1	3
3	0	1	0	0	3
2	1	0	0	0	2
4	0	0	1	1	4
4	1	0	1	0	3
3	0	0	0	1	5
4	0	0	0	1	4
4	0	0	0	1	3
7	0	0	1	1	6

12 month, Site, L probe	12 month treatment site Probe	12 month, site, prove, average	12 month, Site, F rec	12 month, Site, L rec	12 month treatment site Rec
3	4	3.5	1	1	1
5	5	4.5	0	1	1
5	5	4	0	0	0
3	3	3	0	1	1
5	5	3.5	0	0	0
4	4	3.5	0	0	0
5	5	4.5	0	0	0
6	6	5.5	2	0	0
5	6	5.5	0	0	0
5	5	3.5	5	0	0
5	6	5.5	0	0	0
5	5	5	0	0	0
6	5	5.5	0	0	0
4	5	4.5	0	0	0
4	4	4.5	2	1	1
5	4	4.5	0	0	0
3	4	3.5	0	0	0
5	5	4.5	0	1	1
3	4	3.5	0	0	0
4	4	3.5	1	0	0
5	5	4.5	0	0	0
2	2	2	2	2	2
4	4	4	0	0	0
3	3	3	0	1	0
3	4	3.5	0	0	0
4	4	4	1	1	1
5	5	4.5	0	0	0
3	3	3	2	2	2
5	5	4.5	1	0	0
4	4	3.5	1	0	0
4	3	3.5	0	0	0
4	4	4	0	1	0
3	3	3	0	0	0
3	3	3	2	1	1
3	4	3.5	1	2	1

5	5	4.5	0	1	1
4	4	4	0	0	0
5	5	4.5	0	0	0
3	3	2.5	0	0	0
5	4	4.5	0	0	0
5	5	5.5	1	1	1
5	3	4	2	1	2
4	4	3.5	0	0	0
2	3	2	0	0	0
4	4	4	0	1	0
4	4	3.5	1	0	0
6	5	5.5	1	2	1
4	4	4	0	0	0
4	4	3.5	2	1	1
8	6	7	1	0	1

12 month, site, rec, average	12 month, CAL	12 month, Site, F BOP	12 month, Site, L BOP	12 month treatment site BOP	12 month, site, BOP, average	12 month, Adj, F probe
1	4.5	0	0	0	0	3
0.5	5	0	0	0	0	3
0	4	1	1	1	1	3
0.5	3.5	0	1	1	0.5	3
0	3.5	1	1	1	1	3
0	3.5	0	0	0	0	3
0	4.5	1	1	1	1	3
1	6.5	1	0	0	0.5	4
0	5.5	0	1	0	0.5	3
2.5	6	0	1	1	0.5	2
0	5.5	1	1	1	1	6
0	5	0	0	0	0	5
0	5.5	0	1	0	0.5	5
0	4.5	0	1	0	0.5	4
1.5	6	0	0	0	0	4
0	4.5	0	0	0	0	4
0	3.5	1	0	1	0.5	4
0.5	5	0	0	0	0	4
0	3.5	0	0	0	0	3
0.5	4	0	0	0	0	3
0	4.5	0	0	0	0	4
2	4	0	0	0	0	2
0	4	0	0	0	0	3
0.5	3.5	0	0	0	0	2
0	3.5	0	0	0	0	3
1	5	0	0	0	0	6
0	4.5	0	1	1	0.5	3
2	5	1	1	1	1	3
0.5	5	0	0	0	0	5
0.5	4	1	1	1	1	3
0	3.5	0	1	0	0.5	3
0.5	4.5	0	1	1	0.5	4
0	3	0	0	0	0	3
1.5	4.5	0	0	0	0	3
1.5	5	0	0	0	0	3

0.5	5	0	0	0	0	4
0	4	0	1	1	0.5	3
0	4.5	1	1	1	1	4
0	2.5	0	0	0	0	2
0	4.5	0	0	0	0	5
1	6.5	0	1	1	0.5	4
1.5	5.5	0	1	0	0.5	3
0	3.5	0	0	0	0	3
0	2	0	0	0	0	2
0.5	4.5	0	0	0	0	4
0.5	4	0	0	0	0	3
1.5	7	0	1	0	0.5	3
0	4	1	1	1	1	4
1.5	5	1	1	1	1	3
0.5	7.5	1	1	1	1	5

12 month, Adj, L probe	12 month, Adj, F rec	12 month, Adj, L rec	12 month, Adj, F BOP	12 month, Adj, L BOP	18 month, Site, F probe	18 month, Site, L probe	18 month treatment site Probe
2	1	1	0	0	3	3	3
4	0	1	0	0	4	3	3
4	0	0	1	0	2	4	4
3	0	1	0	1	3	4	4
5	2	0	1	1	3	4	4
4	1	0	0	0	2	4	4
6	0	0	1	1	3	4	4
6	0	0	1	0	5	6	6
3	0	0	1	1	4	3	4
4	3	0	0	0	2	5	5
					3	5	5
5	0	0	1	1	4	5	4
6	0	0	0	1	5	6	5
5	2	0	0	0	6	5	6
4	0	0	1	0	4	4	4
4	1	1	0	0	3	4	4
4	0	0	0	0	4	5	4
3	0	0	0	0	4	3	4
5	0	1	1	0	4	4	4
3	0	0	0	0	4	4	4
4	0	0	0	0	3	4	4
5	0	0	0	0	4	4	4
2	2	1	0	0	2	3	2
4	0	0	0	1	3	5	5
1	0	0	0	0	3	3	3
3	0	0	1	1	3	3	3
7	0	1	0	1	4	4	4
3	0	0	1	0	5	5	5
3	1	0	0	1	3	4	4
5	0	0	0	0	5	5	5
3	0	0	1	0	3	3	3
3	0	0	0	1	3	3	3
5	0	0	0	1	3	3	3
3	0	1	0	0	3	4	4
3	1	0	0	0	3	3	3
3	0	3	0	0	3	3	3

5	0	0	0	0	5	5	5
3	0	0	0	1	3	4	4
4	0	0	1	1	4	5	5
3	0	0	1	0	3	4	4
4	0	0	0	1	5	4	5
4	0	1	0	1	5	6	6
4	0	2	0	1	3	3	3
4	0	0	0	0	4	4	4
2	0	0	0	0	3	2	2
4	0	2	1	0	4	4	4
5	1	0	1	1	3	4	4
3	0	0	0	0	7	7	7
4	0	0	1	1	5	3	5
3	0	0	0	0	3	3	3
6	0	0	1	1			

18 month, site, probe, average	18 month, Site, F rec	18 month, Site, L rec	18 month treatment site Rec	18 month, site, rec, average	18 month, CAL
3	1	1	1	1	4
3.5	0	0	0	0	3.5
3	0	0	0	0	3
3.5	1	2	2	1.5	5
3.5	0	0	0	0	3.5
3	0	0	0	0	3
3.5	0	0	0	0	3.5
5.5	1	0	0	0.5	6
3.5	0	0	0	0	3.5
3.5	4	0	0	2	5.5
4	0	1	1	0.5	4.5
4.5	0	0	0	0	4.5
5.5	0	0	0	0	5.5
5.5	0	0	0	0	5.5
4	0	0	0	0	4
3.5	0	0	0	0	3.5
4.5	0	0	0	0	4.5
3.5	0	0	0	0	3.5
4	1	1	1	1	5
4	0	0	0	0	4
3.5	1	0	0	0.5	4
4	0	0	0	0	4
2.5	2	2	2	2	4.5
4	0	0	0	0	4
3	0	0	0	0	3
3	0	0	0	0	3
4	1	0	0	0.5	4.5
5	0	0	0	0	5
3.5	0	0	0	0	3.5
5	0	0	0	0	5
3	1	0	0	0.5	3.5
3	0	0	0	0	3
3	2	2	2	2	5
3.5	0	0	0	0	3.5
3	0	0	0	0	3
3	1	2	1	1.5	4.5

5	0	1	1	0.5	5.5
3.5	0	0	0	0	3.5
4.5	0	0	0	0	4.5
3.5	0	0	0	0	3.5
4.5	0	0	0	0	4.5
5.5	0	0	0	0	5.5
3	1	2	1	1.5	4.5
4	0	0	0	0	4
2.5	0	0	0	0	2.5
4	0	0	0	0	4
3.5	2	0	0	1	4.5
7	0	0	0	0	7
4	1	0	1	0.5	4.5
3	2	1	1	1.5	4.5

18 month, Site, F BOP	18 month, Site, L BOP	18 month treatment site BOP	18 month, site, BOP, average	18 month, Adj, F probe	18 month, Adj, L probe
1	0	1	0.5	2	4
1	0	0	0.5	3	4
1	0	0	0.5	3	4
0	0	0	0	2	3
0	0	0	0	3	5
0	1	1	0.5	2	3
1	1	1	1	4	5
0	1	1	0.5	4	6
1	0	1	0.5	3	3
0	0	0	0	2	4
0	0	0	0	5	5
1	1	1	1	4	4
0	0	0	0	5	7
0	0	0	0	4	4
1	1	1	1	3	2
1	0	0	0.5	3	4
0	0	0	0	3	4
1	1	1	1	4	4
0	1	1	0.5	3	4
1	1	1	1	3	3
0	0	0	0	3	3
1	0	0	0.5	3	5
0	0	0	0	3	3
1	0	0	0.5	3	5
0	0	0	0	0	0
0	0	0	0	3	3
0	0	0	0	3	4
0	0	0	0	2	2
0	1	1	0.5	2	3
0	1	1	0.5	3	3
1	1	1	1	1	2
1	0	1	0.5	3	3
0	0	0	0	2	4
1	1	1	1	3	4
0	0	0	0	3	3
1	0	1	0.5	4	3

1	0	0	0.5	2	3
1	1	1	1	2	3
0	0	0	0	2	2
0	0	0	0	3	3
1	1	1	1	5	4
1	1	1	1	4	6
1	0	1	0.5	3	4
0	1	1	0.5	3	4
0	0	0	0	3	3
0	0	0	0	4	4
1	1	1	1	2	2
1	1	1	1	4	3
1	0	1	0.5	4	4
0	1	1	0.5	3	3

18 month, Adj, F rec	18 month, Adj, L rec	18 month, Adj, F BOP	18 month, Adj, L BOP	24 month, Site, F probe	24 month, Site, L probe
1	1	0	0	4	3
0	1	0	1	4	4
1	0	0	0	3	4
1	2	0	0	3	3
0	1	1	1	3	4
3	0	0	0	2	4
1	0	0	1	5	4
1	0	0	0	6	6
1	0	1	0	3	3
3	0	0	0	2	4
0	1	1	0	4	5
0	0	0	0	5	5
0	0	0	0	4	3
2	1	1	0	5	5
0	0	1	1	4	4
1	1	1	0	3	3
0	0	0	1	4	4
0	1	0	1	4	3
0	0	0	1	4	4
1	1	0	0	3	3
1	1	0	0	4	4
0	0	0	1	3	5
2	1	1	0	3	3
0	0	0	0	3	4
3	3	0	0	3	3
0	0	0	0	3	3
1	0	0	0	3	4
0	0	0	0	4	5
1	0	0	1	2	5
3	0	0	0	3	4
1	0	1	0	3	4
0	0	0	0	4	5
2	1	0	0	3	5
0	0	1	0	2	4
0	0	0	1	3	3
1	3	1	1	4	4

0	0	0	1		
0	0	1	0	4	4
2	1	0	0	3	6
0	0	0	1	3	4
0	0	1	1	3	3
0	1	1	0		
1	2	0	1	3	4
0	0	0	1		
0	0	0	0	3	3
0	0	1	0	4	4
1	1	0	1	3	4
0	0	0	1	6	7
1	0	1	0	4	5
0	0	0	0	2	4

24 month treatment site Probe	24 month, site, probe, average	24 month, Site, F rec	24 month, Site, L rec	24 month treatment site Rec	24 month, site, rec, average
4	3.5	1	1	1	1
4	4	0	0	0	0
4	3.5	0	0	0	0
3	3	1	1	1	1
4	3.5	0	0	0	0
4	3	1	0	0	0.5
4	4.5	0	1	1	0.5
6	6	2	1	1	1.5
3	3	0	0	0	0
4	3	5	1	1	3
5	4.5	0	0	0	0
5	5	0	0	0	0
4	3.5	0	0	0	0
5	5	0	0	0	0
4	4	0	1	0	0.5
3	3	1	1	1	1
4	4	0	0	0	0
4	3.5	0	0	0	0
4	4	1	1	1	1
3	3	0	0	0	0
4	4	1	1	1	1
5	4	0	0	0	0
3	3	1	2	1	1.5
4	3.5	0	0	0	0
3	3	0	0	0	0
3	3	0	1	0	0.5
4	3.5	0	0	0	0
5	4.5	0	0	0	0
5	3.5	1	1	1	1
4	3.5	0	0	0	0
4	3.5	1	0	0	0.5
4	4.5	0	0	0	0
5	4	0	0	0	0
4	3	0	0	0	0
3	3	0	0	0	0
4	4	0	0	0	0

4	4	0	0	0	0
6	4.5	0	0	0	0
4	3.5	0	0	0	0
3	3	1	0	1	0.5
3	3.5	1	2	1	1.5
3	3	0	0	0	0
4	4	0	0	0	0
4	3.5	2	0	0	1
6	6.5	0	1	0	0.5
4	4.5	0	0	0	0
4	3	2	0	0	1

24 month, CAL	24 month, Site, F BOP	24 month, Site, L BOP	24 month treatment site BOP	24 month, site, BOP, average	24 month, Adj, F probe
4.5	1	1	1	1	2
4	0	1	1	0.5	4
3.5	0	0	0	0	1
4	0	0	0	0	3
3.5	0	0	0	0	2
3.5	1	0	0	0.5	2
5	0	1	1	0.5	2
7.5	1	0	0	0.5	5
3	0	0	0	0	2
6	0	0	0	0	3
4.5	0	0	0	0	2
5	0	1	0	0.5	5
3.5	0	0	0	0	4
5	0	0	0	0	4
4.5	0	1	0	0.5	4
4	0	0	0	0	3
4	1	0	1	0.5	3
3.5	1	0	1	0.5	4
5	0	0	0	0	3
3	0	0	0	0	3
5	0	0	0	0	3
4	0	0	0	0	4
4.5	0	1	0	0.5	3
3.5	0	0	0	0	3
3	0	0	0	0	3
3.5	0	0	0	0	2
3.5	0	0	0	0	5
4.5	0	1	1	0.5	3
4.5	0	0	0	0	2
3.5	0	0	0	0	3
4	1	1	1	1	2
4.5	1	1	1	1	4
4	0	0	0	0	4
3	0	0	0	0	3
3	1	1	1	1	3
4	0	0	0	0	3

4	1	1	1	1	3
4.5	1	1	1	1	5
3.5	1	0	0	0.5	3
3.5	0	1	0	0.5	3
5	1	1	1	1	3
3	0	0	0	0	3
4	0	0	0	0	4
4.5	0	1	0	0.5	2
7	0	1	0	0.5	3
4.5	1	1	1	1	3
4	0	0	0	0	2

24 month, Adj, L probe	24 month, Adj, F rec	24 month, Adj, L rec	24 month, Adj, F BOP	24 month, Adj, L BOP	Baseline Radiograph Attempt 1
3	1	1	1	1	3.75
5	0	0	0	0	3.46
3	1	0	0	0	2.88
3	0	1	0	0	3.34
2	1	0	0	0	1.88
3	2	0	1	0	1.81
2	1	1	0	1	3.15
5	2	0	1	0	3.63
3	1	0	0	1	3.28
3	3	1	0	0	5.29
2	1	1	1	0	1.48
4	1	0	1	1	3.33
6	1	0	0	0	4.51
4	2	0	0	0	4.83
5	0	0	0	1	2.23
3	1	1	0	0	4.07
4	0	0	0	0	2.10
4	0	0	1	0	3.41
4	0	0	0	1	3.74
3	1	0	0	0	2.59
4	2	1	0	0	2.93
5	0	0	1	0	3.01
3	2	1	0	0	3.58
4	0	0	0	1	2.35
2	0	0	0	0	6.49
2	0	1	0	0	5.16
4	1	0	0	0	4.96
1	0	0	0	1	3.15
3	2	1	0	1	5.05
4	0	0	0	0	4.32
3	0	0	1	0	4.25
4	0	0	0	1	3.23
4	0	0	0	0	4.53
5	0	0	0	0	1.58
3	0	0	1	1	5.86
3	0	0	0	0	5.61

					5.05
3	0	0	1	1	5.75
5	0	0	1	1	2.62
3	0	0	0	0	2.85
3	0	0	0	1	2.85
					4.66
4	2	2	0	0	5.91
					3.18
2	0	0	0	0	3.52
4	1	0	0	0	3.59
3	2	1	0	1	5.35
3	0	0	0	0	6.76
4	0	1	0	1	3.64
4	0	0	0	0	3.35

Baseline Radiograph Attempt 2	Baseline Radiograph Average	12 Month Radiograph Attempt 1	12 Month Radiograph Attempt 2	12 Month Radiograph Average	24 Month Radiograph Attmept 1
4.02	3.89	3.52	4.37	3.95	3.44
3.25	3.36	3.21	3.45	3.33	3.12
3.12	3.00	2.65	2.93	2.79	2.08
3.79	3.57	3.54	3.78	3.66	3.56
1.97	1.93	1.74	1.84	1.79	1.63
1.98	1.90	1.74	2.45	2.10	1.24
3.25	3.20	2.69	3.77	3.23	2.87
4.81	4.22	4.21	5.02	4.62	4.45
3.03	3.16	3.02	3.26	3.14	3.29
5.63	5.46	5.18	5.47	5.33	5.71
2.78	2.13	2.20	2.79	2.50	2.26
4.21	3.77	4.36	4.24	4.30	4.68
4.67	4.59	4.44	4.68	4.56	4.40
4.67	4.75	4.62	4.93	4.78	4.60
2.60	2.42	2.08	2.31	2.20	2.38
5.61	4.84	3.22	4.80	4.01	3.47
2.97	2.54	2.21	2.39	2.30	2.47
2.22	2.82	3.61	2.94	3.28	3.24
4.84	4.29	3.42	4.26	3.84	3.33
3.25	2.92	2.39	3.10	2.75	1.72
3.34	3.14	2.76	3.20	2.98	2.46
2.77	2.89	2.12	2.80	2.46	1.32
3.73	3.66	3.18	3.70	3.44	3.39
2.13	2.24	2.23	1.83	2.03	2.25
6.62	6.56	6.75	6.49	6.62	6.51
5.04	5.10				5.14
5.34	5.15	4.23	4.33	4.28	4.46
3.87	3.51	2.97	2.74	2.86	3.20
5.05	5.05	5.15	4.43	4.79	4.98
3.82	4.07	3.77	3.41	3.59	4.12
3.29	3.77	3.85	3.26	3.56	4.03
2.81	3.02	3.31	2.36	2.84	3.48
4.18	4.36	4.70	4.28	4.49	4.45
1.92	1.75	1.50	1.75	1.63	1.57
6.31	6.09	5.16	5.81	5.49	5.35
5.78	5.70	4.22	2.80	3.51	4.12

	5.05	4.29	2.24	3.27	
3.44	4.60	5.25	3.73	4.49	4.95
1.76	2.19	2.79	2.16	2.48	2.39
2.67	2.76	2.74	2.79	2.77	2.66
2.69	2.77	3.36	3.11	3.24	3.40
4.12	4.39	4.70	4.20	4.45	3.79
7.06	6.49	6.06	7.02	6.54	6.82
2.46	2.82	3.09	2.41	2.75	
3.26	3.39	2.55	2.43	2.49	2.55
3.77	3.68	4.24	2.82	3.53	3.21
4.99	5.17	5.18	4.50	4.84	5.34
6.24	6.50	5.85	6.11	5.98	6.73
3.36	3.50	3.58	3.35	3.47	3.42
3.25	3.30	3.08	3.14	3.11	3.17

24 Month Radiograph Attempt 2	24 Month Radiograph Average
4.27	3.86
3.17	3.15
2.19	2.14
3.98	3.77
1.78	1.71
3.13	2.19
3.75	3.31
5.20	4.83
3.51	3.40
5.80	5.76
2.73	2.50
4.82	4.75
4.64	4.52
5.04	4.82
2.36	2.37
5.60	4.54
2.76	2.62
2.91	3.08
4.46	3.90
3.47	2.60
3.31	2.89
2.66	1.99
3.87	3.63
2.14	2.20
6.43	6.47
5.06	5.10
4.57	4.52
3.31	3.26
4.77	4.88
3.56	3.84
3.32	3.68
2.73	3.11
4.23	4.34
1.80	1.69
5.64	5.50
2.78	3.45

3.28	4.12
1.88	2.14
2.89	2.78
2.59	3.00
4.09	3.94
7.11	6.97
2.52	2.54
3.16	3.19
5.22	5.28
6.15	6.44
3.38	3.40
3.17	3.17